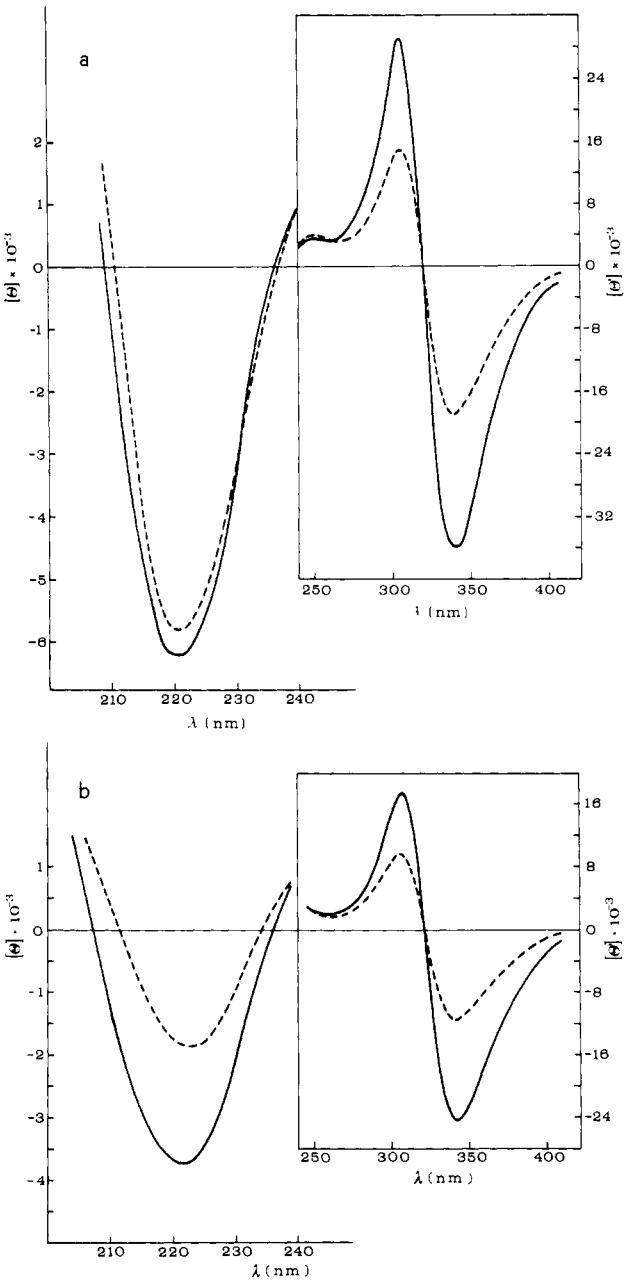

Figure 4. (a) CD spectra of poly(L-glutamic acid) containing 36 mol % of azo groups before (—) and after (---) irradiation, in aqueous solution at pH 4.8. (b) The same spectra as in (a) recorded at pH 6.5.

However, the plotting of $[\Theta]_{220}$ vs. pH indicates (Figure 3) that the order–disorder conformational transition occurs with different pK values for the dark-adapted sample (pK = 6.8) and the irradiated one (pK 6.3).[7] This means that the β structure has different stability when the 36 mol % of azo groups are either all trans or 70% trans and 30% cis. Accordingly, irradiation in the critical range of pH between the two pK values should produce a conformational change of the polypeptide chain.

The occurrence of this change is shown by the CD spectra, before and after irradiation at 369 nm, recorded at pHs 4.8 and 6.5, respectively (Figure 4a,b). At pH 4.8, where the β structure is very stable and not affected by azo side-chain photoisomerizations, the 30% trans → cis photoconversion produces a strong decrease in the ellipticity of the dichroic bands in the azo π–π^* absorption range (350 nm), but no effect is observed in the peptide region (Figure 4a). When the irradiation is carried out at pH 6.5, intermediate between the two above pK values, an analogous variation of the CD spectrum is observed in the azobenzene main absorption region. Moreover, a contemporary remarkable decrease of the ellipticity of the 220-nm band, associated with the β structure, can be observed (Figure 4b). The lack of variation in this region by irradiating at pH 4.8 excludes possible contributions of the azobenzene groups.

The conformational change induced by light is completely reversible, exposition of the polypeptide alternately to light and dark conditions reproducing exactly the two expected CD spectra, as observed in photoregulated biological processes.[8]

Systematic investigation in due course about the effect of solvent, irradiation wavelength, and azobenzene content and distribution should permit us to obtain larger conformational modifications.

(7) The same pK values were also found by plotting the amplitude of the couplet at ~350 nm vs. pH. This use of the azo chromophore as conformational probe is consistent with the direct relationship between side chains CD bands and main chain conformation.

(8) Erlanger, D. F. *Annu. Rev. Biochem.* **1976**, *45*, 267–283.

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Chiral Recognition by Small Biological Molecules. Resolution of Helicenes on Silica Gel Coated with Riboflavin

Sir:

Recent work has demonstrated that a wide range of optically active compounds shows significant differences in their distribution between two phases, one of which is chiral and can therefore be readily resolved, e.g., by gas[1] or liquid chromatography.[2] Such chromatographic experiments, when carried out with biological substances as either the resolving or the resolved species, have particular interest, as they might have relevance for the understanding of chiral discrimination in biological systems.

Optically active natural polymers can be used more or less successfully for the separation of enantiomers. It will suffice here to mention the classical resolution of Troeger's base on lactose[3] and the more recent separations achieved on starch columns[4] and on modified (triacetylated) cellulose.[5]

For small molecules, protein amino acids are examples of compounds which, derivatized as, e.g., *N*-acyl dipeptide esters,[6] *N*-acyl amino acid amides,[7] or metal complexes,[8] show chiral recognition as stationary (or mobile) phases in either gas or liquid

(1) E. Gil-Av, B. Feibush, and R. Charles-Sigler, *Tetrahedron Lett.*, 1009 (1966), and subsequent papers.
(2) (a) I. S. Kroll, *Adv. Chromatogr. (N.Y.)*, **16**, 175 (1977); (b) E. B. Kyba, K. Koga, L. R. Sousa, M. G. Siegel, and D. S. Cram, *J. Am. Chem. Soc.*, **95**, 2692 (1973), and subsequent papers; (c) J. N. LePage, W. Lindner, G. Davies, D. E. Seitz, and B. L. Karger, *Anal. Chem.*, **51**, 433 (1979); (d) W. H. Pirkle and D. W. House, *J. Org. Chem.*, **44**, 1957 (1979).
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Table I. Resolution of Carbohelicenes by High-Pressure LC on Silica Gel Coated with Riboflavin and with CH_2Cl_2/n-Hexane as Eluant[a]

	5% coating			8% coating					
	50% CH_2Cl_2			20% CH_2Cl_2			50% CH_2Cl_2		
helicenes	k_1'[b]	k_2'[b]	r[c]	k_1'[b]	k_2'[b]	r[c]	k_1'[b]	k_2'[b]	r[c]
[6]				2.4	2.4	1.00			
[7]				3.24	3.41	1.052			
[8]				3.62	3.87	1.069			
[9]	0.97	0.97	1.00	4.83	5.20	1.077	0.72	0.72	1.00
[10]	1.19	1.27	1.067	6.46	7.02	1.087	0.81	0.86	1.062
[11]	1.32	1.40	1.061	7.52	8.23	1.094	0.89	0.97	1.090
[12]	1.60	1.72	1.075	9.59	10.46	1.091	1.05	1.14	1.086
[13]	1.85	2.02	1.092	11.48	12.70	1.106	1.25	1.37	1.096
[14]	1.93	2.12	1.098	11.85	13.39	1.130	1.37	1.52	1.109

[a] See text for other chromatographic conditions. [b] k_1' and k_2' = capacity factors of the first and second enantiomeric peaks, respectively. [c] $r = k_2'/k_1'$.

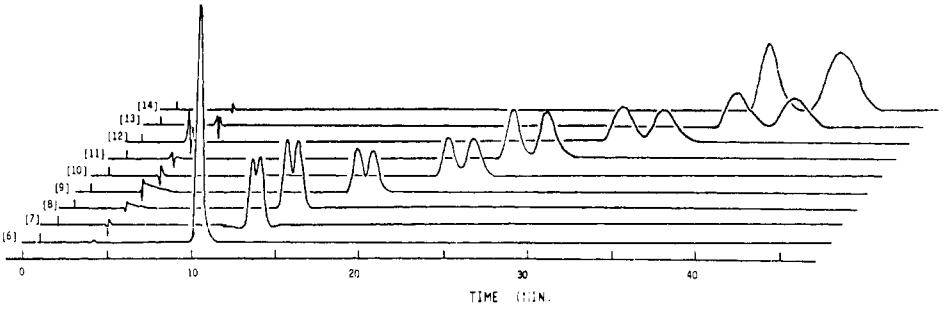
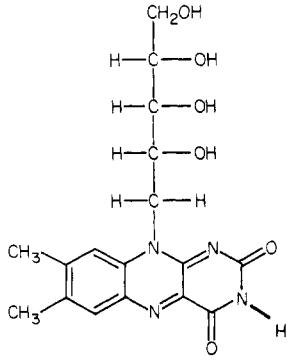


Figure 1. Resolution of carbohelicenes by high-pressure liquid chromatography on silica gel coated with riboflavin.

chromatography. It is of interest to note that the chiral discrimination manifested to a practically absolute degree by enzymes is refound, at least in a rudimentary fashion, in the simple building blocks of the complex polypeptidic structures.

Molecular association leading to chiral recognition can involve various types of interactions, such as hydrogen bonding, charge-transfer complexation, and coordination to metals, or combinations thereof. It was reported several years ago that charge-transfer complexation can be employed for the efficient resolution of helicenes by high-pressure liquid chromatography (high-pressure LC) on silica gel to which optically active 2-[[(2,4,5,7-tetranitro-9-fluorenylidene)amino]oxy]propionic acid (TAPA) is linked covalently.[9,10] There exist a large number of biological molecules, such as the nucleotides, the nucleosides, and the flavins, which have certain general structural features recalling those of TAPA. Thus, riboflavin[12] and TAPA have both a tricyclic



system, known to form charge-transfer complexes with different compounds, in particular aromatic hydrocarbons, and a side chain attached to the central ring and containing one (or more) asymmetric center(s).

In this communication, we report on the resolution of [6]- to [14]-carbohelicenes on silica gel coated with riboflavin. A known amount of riboflavin dissolved in water was coated on 5-μm Lichrosorb Si 100 (Merck, Darmstadt, Germany),[12] which after drying was filled into a 20 × 0.46 cm stainless-steel column by the conventional slurry packing method. The column with 8% w/w[13] riboflavin gave a reduced plate height of 5.4 for naphthalene with 20% methylene chloride/n-hexane. The eluate was detected by UV; for further chromatographic conditions, see Table I and Figure 1.

The retention times and resolution factors (r) are listed in Table I. It can be seen that, generally speaking, there is a tendency for the r values to increase with the number of rings. The correlation of this observation, also found for TAPA,[9] with the structure of the solutes and other parameters involved awaits further studies. It is known that charge-transfer complexation of a molecule is related to its ionization potential, and it has indeed been established that there is a good correlation between the ionization potential of a series of aromatic molecules and their association constants with riboflavin in ethanol.[11] However, the capacity factors of naphthalene, phenanthrene, fluoranthene, pyrene, and anthracene on riboflavin (8% column, 0.14, 0.33, 0.58, 0.54, and 0.33, respectively) do not correlate as well with the corresponding ionization potentials (8.10, 8.09, 7.72, 7.55, and 7.37 eV).[11] Other factors, such as molecular size, would appear also to play a role in the adsorption on silica gel/riboflavin. Another interesting feature is that the resolution process can be affected with an aprotic, apolar solvent as the mobile phase; no sttudies of complexation with riboflavin have, thus far, been carried out in such media.[11]

The order of emergence has been established by coinjection of (−)-[10]- and (−)-[14]-helicene with corresponding racemic mixtures, as P (+) precedes the M (−) isomer, i.e., the reverse of that found on (R)-(−)-TAPA.[9]

Similar experiments have also been carried out with silica gel coated with adenosine and adenylic acid, respectively, as will be

(9) F. Mikes, G. Boshart, and E. Gil-Av, *J. Chromatogr.*, **122**, 205 (1976).
(10) H. Numan, R. Helder, and H. Wynberg, *Recl. Trav. Chim. Pays-Bas*, **95**, 211 (1976).
(11) M. A. Slifkin, "Charge Transfer Interactions of Biomolecules", Academic Press, London, 1971, p 132.
(12) In situ coating was not possible because of the low solubility of riboflavin in organic solvents.
(13) The content of riboflavin was determined by microanalysis.

reported in full elsewhere. We wish, however, to mention that, as expected, in these cases stereoselectivity has also been observed.

These results demonstrate the usefulness of the chromatographic approach to the study of the complexation and stereoselectivity of biological molecules. The continuation of such studies should permit us to recognize the structural parameters and, possibly, the intermolecular interactions responsible for chiral recognition. Vice versa, these investigations should also lead to the design of novel synthetic molecules for the efficient resolution of optical isomers.

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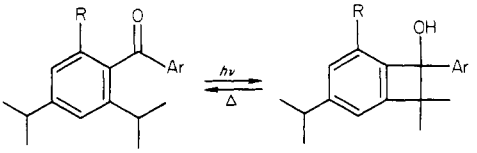
Sterically Promoted Benzocyclobutenol Formation from 2,4,6-Triisopropylbenzophenone Photolysis[1]

Sir:

Our persistent interest in high-energy compounds[2] urges us to search for a new system for the chemical storage of solar energy.[3] Benzocyclobutenols, which can be prepared by the photocyclization of particular 2-alkyl-substituted phenyl ketones,[4] are highly strained, and their thermal back reactions to the parent ketones are considerably exothermic.[5] Besides the practical interest in the benzocyclobutenol molecule as a potential for solar energy storage, its formation reaction is deeply related to the recent considerable controversy in the mechanism of photoenolization of 2-alkyl-substituted phenyl ketones.[4c,e,7] Previously, Matsuura and Kitaura found a quantitative reversible photocyclization of 2,4,6-triisopropylbenzophenone (**1a**) to the corresponding benzocyclobutenol **2a** (eq 1).[4a] We have undertaken a quantitative study of this reaction and found that steric effects are crucial for understanding this reaction.



(1)

1a, R = *i*-Pr, Ar = Ph

2a, R = *i*-Pr, Ar = Ph

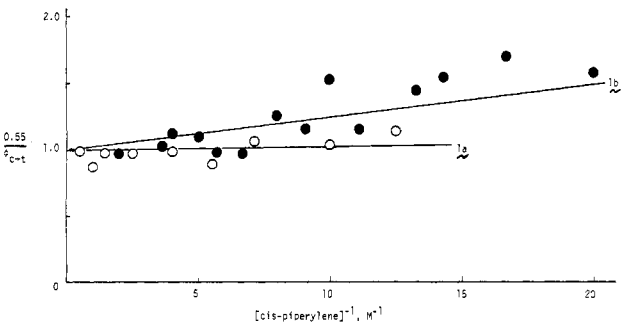


Figure 1. Photosensitized cis–trans isomerization of *cis*-piperylene in benzene by 0.05 M 2,4,6-triisopropylbenzophenone (**1a**) (○) and 0.05 M 2,4,6-trimethylbenzophenone (**1b**) (●).

Table I. Excited-State Properties of 2,4,6-Triisopropylbenzophenone (**1a**) and 2,4,6-Trimethylbenzophenone (**1b**)[a]

compd	Φ_{ST}	Φ_{CB}	$k_q\tau_T$, M^{-1}	τ_T, ns	E_S, kcal/mol	E_T, kcal/mol
1a	1.01 ± 0.04[b]	0.60	450 ± 30[d]	90 ± 6[e]	~75[f]	68.7[g]
1b	1.07 ± 0.15[b]	0.002[c]	35 ± 20[b]	7 ± 4[e]	~75[f]	~69[h]

[a] All the photokinetic data were obtained in benzene at 25 °C. [b] Determined from the photosensitized cis-trans isomerization of *cis*-piperylene (Figure 1).[21] [c] Reference 4a. [d] Slopes of linear Stern–Volmer plots with 2,5-dimethyl-2,4-hexadiene as quencher. [e] $k_q = 5 \times 10^9$ M^{-1} s^{-1} in benzene. [f] Estimated from the absorption spectra. [g] Reference 22. [h] Estimated from the phosphorescence spectrum in ethanol at 77 K.[23]

The quantum yield of the cyclobutenol formation (Φ_{CB}) for the reaction **1a** → **2a** was quite high and independent of the polarity or the hydrogen-bonding ability of the solvents employed, i.e., Φ_{CB} = 0.60 (benzene), 0.62 (*n*-hexane), 0.42 (cyclohexane), 0.60 (methanol), 0.77 (acetonitrile), 0.55 (Freon-11), and 0.52 (*n*-propyl bromide) at 25 °C.[8] This finding is remarkable, since Φ_{CB} is less than 0.1 in all the benzocyclobutenol formation reactions so far reported[4] with only one exception, γ-2,4,6-tetramethylvalerophenone (**3**), where Φ_{CB} is 0.21 in benzene containing 3 M dioxane.[4e]

Wagner and Scaiano have recently reported that 2-alkyl-substituted phenyl ketones such as 2-methylacetophenone (**4**),[7a,d] 2,4,6-triisopropylacetophenone (**5**),[4h] 2-methylbenzophenone (**6**),[4e] or **3**[4e] have two kinetically distinct triplets, i.e., a long-lived (>10 ns) and a short-lived (<1 ns) triplet, and that the overall triplet quantum yield was far less than unity in each ketone except **6**. In the cases of **3** and **5**, the short-lived triplets lead to the formation of the corresponding benzocyclobutenols, which are only inefficiently quenched by diene.[4e,h] In sharp contrast to these ketones (**3**–**6**), we estimated by the photosensitized cis–trans isomerization of *cis*-piperylene in benzene (Figure 1) that **1a** had only a long-lived triplet with the intersystem crossing yield Φ_{ST} = 1.01 ± 0.04.[9] Furthermore, the formation of **2a** was efficiently quenched by

(1) Photoinduced Reactions, part 121.
(2) Ito, Y.; Matsuura, T.; Yokoya, H. *J. Am. Chem. Soc.* **1979**, *101*, 4010–4011. Ito, Y.; Matsuura, T.; Kondo, H. *Ibid.* **1979**, *101*, 7105–7107.
(3) Laird, T. *Chem. Ind. (London)* **1978**, 186–193. Wrighton, M. S. *Chem. Eng. News* **1979**, *57*, (Sept 3), 29–47.
(4) (a) Kitaura, Y.; Matsuura, T. *Tetrahedron* **1971**, *27*, 1597–1606. (b) Matsuura, T.; Kitaura, Y. *Ibid.* **1969**, *25*, 4487–4499. (c) Carrê, M. C.; Viriot-Villaume, M. L.; Caubère, P. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2542–2549. (d) Viriot-Villaume, M. L.; Carrê, C.; Caubère, P. *Ibid.* **1979**, 1395–1403. (e) Wagner, P. J. *Pure Appl. Chem.* **1977**, *49*, 259–270. (f) Arnold, B. J.; Mellows, S. M.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 401–409. (g) Heine, H. G. *Justus Liebigs Ann. Chem.* **1970**, *732*, 165–180. (h) Wagner, P. J., private communication.
(5) The following thermochemical and kinetic data were found for the thermal decomposition **2a** → **1a**: ΔH = −20.08 kcal/mol (solid) (Inagaki, S.; Murata, S.; Sakiyama, M.; Ito, Y.; Umehara, Y.; Hijiya, T.; Matsuura, T. 15th Symposium on Thermochemical Measurement, Kanazawa, Oct. 1979; Abstr. p 150); $k = 3.52 \times 10^{-5}$ s^{-1} at 130.1 °C; E_a = 28.6 ± 1 kcal/mol; log A = 11.0 ± 1; $\Delta H^{\ddagger}$ = 27.8 ± 1 kcal/mol; $\Delta S^{\ddagger}$ = −10.7 ± 4 eu. The kinetic data were obtained in phenyl ether solvent (119–139 °C). Note that ΔH of quadricyclane to norbornadiene is exergonic only by 9.94 kcal/mol (gas).[6]
(6) Pedley, J. B.; Rylance, J. "Sussex—N. P. L. Computer Analyzed Thermochemical Data: Organic and Organometallic Compounds", University of Sussex: Brighton BN 19 Q, 1977.
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(8) In some of the good hydrogen-donating solvents, however, the quantum yield was significantly lower and as yet unidentified byproducts were formed probably as a result of a competitive intermolecular hydrogen abstraction, i.e., Φ_{CB} = 0.19 (ethyl ether), 0.19 (2-propanol), and 0.27 (ethanol). The type II elimination reaction of valerophenone in benzene was used as a standard for the quantum yield measurements. In all experiments throughout the present study, 0.1 M ketone solutions were degassed and irradiated on a merry-go-round apparatus by 313-nm light. The benzocyclobutenols were analyzed by high-pressure liquid chromatography (hexane–ethyl acetate, Jascosil SS-05).
(9) Because of the relatively long triplet lifetimes of **1a** and **1b** and of the experimental limitations in our VPC analysis, τ_T determined by this sensitization study was subject to large errors, and we cannot rule out the possibility that another longer lived triplet state of **1a** and **1b** might also be involved.[10]
(10) Wagner, P. J. *J. Photochem.* **1979**, *10*, 387–399.

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